RECEPTOR BIOCHEMISTRY AND METHODOLOGY, VOLUME 1

Series Editors: J. Craig Venter and Len C. Harrison

MEMBRANES, DETERGENTS, AND RECEPTOR SOLUBILIZATION

Editors
J. Craig Venter
Len C. Harrison

Alan R. Liss, Inc., New York

MEMBRANES, DETERGENTS, AND RECEPTOR SOLUBILIZATION

Editors

J. Craig Venter
Department of Molecular Immunology
Roswell Park Memorial Institute
Buffalo, New York

Len C. Harrison
Department of Diabetes and Endocrinology
The Royal Melbourne Hospital
Victoria, Australia

ALAN R. LISS, INC., NEW YORK

Address all Inquiries to the Publisher
Alan R. Liss, Inc., 150 Fifth Avenue, New York, NY 10011

Library of Congress Cataloging in Publication Data
Main entry under title:

Membranes, detergents, and receptor solubilization.

(Receptor biochemistry and methodology ; v. 1)
Includes index.
1. Cell membranes. 2. Cell receptors. 3. Solubilization. 4. Surface active agents. I. Venter, J. Craig.
II. Harrison, Len. III. Series.
QH601.M4819 1983 574.87'5 83-16271
ISBN 0-8451-3700-X

Contributors

Barbara D. Boyan [47]
Department of Periodontics and Biochemistry, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284

Andreas Chrambach [35]
Laboratory of Theoretical and Physical Biology, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20205

Yvonne Clement-Cormier [47]
Department of Pharmacology, The University of Texas Health Science Center at Houston, Houston, TX 77030

Cyril D. Curtain [177]
CSIRO Division of Chemical Technology, Clayton, Victoria 3168, Australia

Michael P. Czech [161]
Department of Biochemistry, University of Massachusetts Medical Center, Worcester, MA 01605

Darrell Doyle [1]
Department of Biological Sciences, State University of New York at Buffalo, Buffalo, NY 14214

Mahmoud F. El-Refai [99]
Department of Physiology and Biophysics, University of Southern California School of Medicine, Los Angeles, CA 90701

Barry H. Ginsberg [65]
Departments of Biochemistry and Internal Medicine, Diabetes-Endocrinology Research Center, University of Iowa, and Veterans Administration Medical Center, Iowa City, IA 52242

Larry M. Gordon [177]
Rees-Stealy Research Foundation, San Diego, CA 92101

Robert J. Gould [65]
Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Len C. Harrison [xi]
Department of Diabetes and Endocrinology, The Royal Melbourne Hospital, Parkville, Victoria 3050, Australia

The number in brackets is the opening page number of the contributor's article.

Fusao Hirata [27]
Laboratory of Cell Biology, National Institute of Mental Health, NIH, Bethesda, MD 20205

Leonard M. Hjelmeland [35]
Laboratory of Vision Research, National Eye Institute, NIH, Bethesda, MD 20205

Anh Van Le [1]
Department of Biological Sciences, State University of New York at Buffalo, Buffalo, NY 14214

Paul F. Pilch [161]
Department of Biochemistry, Boston University School of Medicine, Boston, MA 02118

Abbas Rashidbaigi [119]
Department of Pharmacology, University of Wisconsin Medical School, Madison, WI 53706

Phoebe E. Roeder [119]
6789 Alamo Way, La Mesa, CA 92041

Arnold E. Ruoho [119]
Department of Pharmacology, University of Wisconsin Medical School, Madison, WI 53706

William L. Strauss [85]
Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20205

J. Craig Venter [xi]
Department of Molecular Immunology, Roswell Park Memorial Institute, Buffalo, NY 14263

Glenn M. Ward [109]
University of Melbourne Department of Medicine, The Royal Melbourne Hospital, Parkville, Victoria 3050, Australia

Contents of Volumes 2 and 3

Volume 2: Receptor Purification Procedures

Volume 3: Molecular and Chemical Characterization of Membrane Receptors

Preface

Cell hormone and neurotransmitter receptors are involved in the control and/or modulation of virtually every cellular and physiological process known. Changes in hormone receptors are implicated in major diseases including heart disease, hypertension, allergic respiratory diseases, metabolic disorders such as diabetes, and cancer. The recent molecular breakthroughs in the area of hormone and neurotransmitter receptor research have resulted from an interdisciplinary approach with the application of technologies derived from biochemistry, immunology, pharmacology, molecular biology, biophysics, and medicine.

The receptor field is undergoing a period of rapid advancement with a tremendous growth in the number of studies at the molecular level. This transition from receptor analysis using pharmacological techniques and radioligand binding studies toward the molecular resolution of the receptor molecules and their effector proteins requires working with a broad repertoire of new methodologies and techniques.

Receptor Biochemistry and Methodology is a new series devoted to the molecular advances involved in understanding the structure and biochemical basis of receptor action as well as to detailing the necessary methodologies.

The first three volumes are methodological reviews of approaches and technologies required by researchers involved in the isolation, purification, and biochemical characterization of cell surface proteins. Future volumes will deal in depth with single areas including Monoclonal Antibodies (Volume 4) and Radiation Inactivation (Volume 5), or will be devoted to the molecular basis of hormone and neurotransmitter action, with individual volumes on the structure, function, pharmacology, and biochemistry of individual receptors or effector proteins.

In Volume 1, **Membranes, Detergents, and Receptor Solubilization,** some of the essentials for undertaking biochemical studies of receptors are provided. The first two chapters review cell membrane structure and function and the interactions that occur between proteins and lipids. The next three chapters detail the various strategies employed in the purposeful disruption of the hybrophobic forces that hold proteins in place in the membrane. Chapters 6 to 10 describe various means of identification and assay of solubilized receptors, including photoaffinity labeling, affinity cross-linking, sulfhydryl group modification, and protein iodination. Finally, Chapter 11 on electron spin resonance spectroscopy of membranes deals quantitatively with changes in the membrane lipid environment and its effect on integral membrane proteins.

The contents of Volumes 1 to 3 reflect a strong prejudice, developed by us both in the course of our respective receptor purification studies, that one key to success in receptor purification and structural analysis is to utilize many parallel approaches to insure, for example, that the isolated proteins are indeed those desired.

We feel fortunate in obtaining a unique collection of authors who are leaders in their respective fields and thank them for their help in making this series a reality. We also thank Paulette Cohen and the staff of Alan R. Liss, Inc. for their help and advice.

We believe that the reader will find that the articles are not only excellent reference sources but also extremely useful at the laboratory bench. We feel that the information contained in these volumes will be essential to researchers undertaking the molecular characterization of receptors or other integral membrane proteins. In addition, we hope that the reviews and discussions will interest researchers in biochemistry, immunology, pharmacology, physiology, biophysics, and cell and molecular biology as well as researchers and others who deal with the vast array of clinical disorders effected by or controlled through receptors.

J. Craig Venter
Len C. Harrison

Contents

Membranes, Detergents, and Receptor Solubilization, pages 1–25

1

General Theory of Membrane Structure and Function

Anh Van Le and Darrell Doyle

Department of Biological Sciences, State University of New York at Buffalo, Buffalo, New York 14214

INTRODUCTION

The general topography of the mammalian cell consists of a complex membrane system which includes the nuclear membrane, smooth and rough endoplasmic reticulum, Golgi and Gerl structures [Novikoff, 1976], lysosomes, mitochondria, and a variety of poorly defined intracellular vesiclelike structures, all enclosed within the plasma membrane. All cells must bring biologically important molecules from the cell exterior to the interior and vice versa. Specific transport systems carry small molecules (ie, metabolites such as anions, amino acids, simple sugars, and other small molecular weight materials) across the membranes. Larger molecules such as plasma proteins, toxins, viruses, etc, are transported or ingested by other systems. Many of the physiological properties of cells are, therefore, intimately linked to membrane structures. In order to achieve a satisfactory understanding of any biological system, a detailed molecular composition and structure of that system should be known. We will, therefore, attempt to summarize what is presently known or accepted about the molecular organization of membranes in mammalian cells. Then we will present studies which we feel represent the better approaches toward an understanding of membrane structure-function relationships.

GENERAL ASPECTS OF MEMBRANE STRUCTURE

The principal components of membrane are lipids, proteins, and carbohydrates. In erythrocytes, approximately 52% of the membrane mass is protein, 40% is lipid, and 8% is carbohydrates [Dodge et al, 1963; Rosenberg and Guidotti, 1968; Winzler, 1969]. About 7% of this sugar is carried by glycolipids, the rest by membrane proteins [Winzler, 1969; Sweeley and Dawson, 1969; Jamieson and Greenwalt, 1971]. The composition of the erythrocyte membrane reflects the general composition of most cell membranes. However, different cell membranes vary in the proportion of their membrane components, some of which could result from growth rate [Hynes, 1976; Gahmberg, 1977; McNutt et al, 1973], state of differentiation [Eisen et al, 1977; Geiduscheck and Singer, 1979; Chan and Oliver, 1976], viral infection [Nicolson, 1976; Hynes, 1976; Steiner and Steiner, 1978; Noonan, 1978; Lausch and Rapp, 1978], drug administration to [Orrenius and Ericsson,

1966], or nutritional status of, the cell [Awad and Spector, 1976; Engelhard et al, 1976; Hopkins and West, 1977; Burns et al, 1979].

Lipids

It is now almost universally accepted that lipids constitute the structural framework of the biological membrane. The familiar bilayer arrangement, in which the hydrocarbon chains of the fatty acids of each layer are in close opposition, reflect the combined effect of two major noncovalent interactions: hydrophobic and hydrophilic [Singer, 1971; Kauzmann, 1959]. When a pure species of phospholipids is introduced into a neutral aqueous environment, the nonpolar fatty acyl chain is sequestered together away from contact with water, thereby maximizing hydrophobic interactions. The ionic groups are in direct contact with the aqueous phase at the exterior surface of the bilayer, in favor of a more hydrophilic environment. These characteristics, as we will see later, place restrictions on the distribution of proteins within the lipid bilayer.

Lipids are usually classified in three categories: phospholipids, neutral lipids, and glycolipids. Phospholipids are by far the most abundant in the membrane. Commonly found in mammalian cells are phosphatidylethanolamine (PE), -serine (PS), -choline (PC), -inositol, and sphingomyelin (SP). Other phospholipids such as plasmalogens, lysophosphatides, phosphatidic acid, and cardiolipins are occasionally found in cell membranes and represent a minor proportion of the total phospholipids. Predominant among the neutral lipids is cholesterol, whereas the glycerides and cholesteryl esters are present in negligible amounts. Unlike the phospholipids and cholesterol which compose the bulk of the membrane lipids, the glycolipids represent only a minor portion. In general, they can be divided into two groups, the glycosphingolipids and the glycoglycerolipids. Glycosphingolipids are present in membranes of most animal tissues, whereas the glycoglycerolipids are found mainly in brain [Sweeley and Siddiqui, 1977], testis and sperm cells [Kornblatt et al, 1972; Ishizuka et al, 1973]. The neutral glycosphingolipids (containing neutral sugars), the gangliosides (containing sialic acid), and the sulfateglycosphingolipids (containing sulfate ester groups on the carbohydrate moiety) make up the subgroups of the glycosphingolipids.

The lipid composition is not always the same for different membrane systems of the same cell (Table I). Rat liver plasma membrane generally contains most of the glycolipids and shows the highest content of cholesterol [Van Hoeven and Emmelot, 1972]. The rough endoplasmic reticulum membrane contains a higher proportion of phospholipids than plasma membrane but very little cholesterol. Golgi membrane is intermediate between the rough endoplasmic reticulum and the plasma membrane. This intermediate composition is in agreement with the postulate that Golgi membrane is a transitional membrane undergoing modification to form new plasma membrane [Doyle and Baumann, 1979; Doyle et al, 1978a,b; Farquhar, 1978; Jamieson and Palade, 1977; Palade, 1975]. Differences in the composition of various classes of lipids are also observed in similar membranes of various species. The relative composition of cholesterol and phospholipid (expressed as a percentage of total lipids) is approximately the same in membrane of rat, porcine, bovine, and human erythrocytes. The latter two show the lowest content of glycolipids, whereas porcine shows the highest. Moreover, there is variation in the phospholipid composition itself. Bovine erythrocytes have virtually no PC but a high content of SP. In contrast, rat erythrocytes have high PC and low SP. Human and porcine erythrocytes show intermediate values for both PC and SP. These data suggest that in the case of the erythrocyte membrane, PC can be replaced by SP without significant changes in membrane biological function. The variation in phospholipid composition is also found in different membrane systems of rat liver cells. Most significantly, PC represents the highest

TABLE I. Lipid Composition of Various Membranes*

Membrane	Phospholipids[a]										Sterol	Glycolipids
	PC	PE	PS	PI	PA	SP	CL	LPC	LPE	Total		
Human erythrocytes[b]	19	22	6	6	n.r.	2.0	n.r.	n.r.	n.r.	73	25	<5
Bovine erythrocytes[b]	0	21	8		n.r.	41	n.r.	n.r.	n.r.	70	30	<5
Porcine erythrocytes[b]	16	26	5	0.6<	n.r.	16	n.r.	n.r.	n.r.	63.6	28	12–14
Rat erythrocytes[c]	32	14.5	7.2	2.3	0.2<	8.6	n.r.	2.6	n.r.	67.4	24.7	8.3
Rat liver plasma[d] membrane	20	11.5	5.1	4.2	2.5	10	Trace	1.9	n.r.	55.2	22	8
Nuclear membrane	52.2	19.3	3	7.3	0.05<	2.7	0	1.3	0	86.6	-	-
Golgi	24.5	9.7		4.7	n.r.	6.7	n.r.	3.2	3.4	54.5	12	-
Rough endoplasmic reticulum	51.8	15.8	2.8	7.6	0.85	3.1	n.r.	4	0	85.95	6.5	-
Lysosomal	23.4	12.4	6.2	6.2	4.7	22.8	4.7	0	n.r	81.4	22	-

*Values expressed as per cent of total lipids.

[a]PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PA, phosphatidic acid; SP, sphingomyelin; CL, cardiolipin; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PG, phosphatidylglycerol; n.r., not reported.

[b]Calculated from data of Hanahan [1969].

[c]Calculated from data of Nelson [1967] and Rouser et al [1968].

[d]Calculated from McMurray and Magle [1972], van Hoeven and Emmelot [1972], Glaumann and Dalner [1968], Keenan and Morre [1970].

proportion of phospholipid in the nuclear and rough endoplasmic reticulum membrane. Lysosomal membrane has the highest proportion of sphingomyelin and a small proportion of cardiolipin. The latter generally is found in mitochondrial membranes [McMurray and Magee, 1972].

Proteins

Although lipids constitute the matrix of the membrane, the specifities of biological activities of the cell membrane reside primarily in the proteins interacting with the lipid bilayer. As might be expected, the nature and type of proteins associated with the various membranes vary widely. For example, cells of hepatocyte origin in tissue culture have at least 100 proteins and glycoproteins externally oriented on their plasma membrane [Tweto et al, 1976; Baumann and Doyle, 1979; Doyle and Le, 1981]. Membrane proteins can be generally classified as peripheral or integral proteins. Peripheral proteins can be released from the membrane by mild treatments such as an increase in ionic strength, heavy metals, chelating agents, low concentration of urea, etc. They can be located on both the inner and outer surface of the plasma membrane. These proteins include actin/myosin filaments, fibronectin, and spectrin, which are members of the cytoskeletal and exoskeletal systems of the cell [Branton et al, 1981]. Peripheral proteins include proteins involved in cell-cell contact and recognition such as collagen, mucoproteins, collagen binding proteins, etc. These proteins are usually associated with the membrane by ionic interaction with the polar head groups of phospholipids, or by covalent or noncovalent interaction with membrane proteins or oligosaccharide components of glycolipids. Another characteristic of peripheral proteins is their relative solubility in neutral aqueous buffer once they are dissociated free of lipids.

Integral proteins generally require much more drastic treatment such as detergents, organic solvents, chaotropic agents, bile salts, etc, to release them from the lipid bilayer in which they are embedded. They often are highly insoluble and will aggregate in neutral aqueous buffer if completely free of lipids. Integral proteins include those that traverse the lipid bilayer such as glycophorin, histocompatibility antigens, viral glycoproteins, acetylcholine receptor, $(NA^+ + K^+)$ ATPase, etc, and those that partly penetrate the bilayer (ie, cytochrome b5 and adenylate cyclase). In addition, integral membrane proteins can also be distinguished by differences in their secondary modifications. They can be either nonglycoconjugated proteins or glycoproteins. The carbohydrates found on the glycoproteins are preferentially exposed to the external surface of the plasma membrane. Although many of the receptors for biologically active molecules are membrane-associated glycoproteins, the actual role of the carbohydrate moiety on these important proteins is not yet fully understood.

Insertion of Proteins Into the Membrane

The decision as to whether a protein is to be secreted or to remain associated with the membrane is genetically defined. The information is encoded in the mRNA which codes for the protein product. Protein synthesis begins on unattached polysomes. Membrane-associated proteins, like secretory proteins, are synthesized with an NH_2-terminal extra piece, the "signal sequence" or leader sequence [Milstein et al, 1972; Kreil, 1981; Sabatini et al, 1982]. Upon emergence of the signal sequence from the large ribosomal subunit, the ribosomal complex specifically makes contact with the membrane, through an integral membrane receptor protein and a signal recognition particle [Meyer et al, 1982; Meyer and Dobberstein, 1980a,b]. The nascent protein is then translocated across the endoplasmic reticulum membrane. A protease on the noncytoplasmic face of the rough endoplasmic reticulum can remove the signal peptide. However, in certain cases, it appears that the leader sequence need not be at the NH_2-terminal end, and its cleavage is also not a prerequisite for translocation. Ovalbumin, a major secretory product of the chicken oviduct, is made without a transient NH_2-termi-

nal leader peptide [Gagnon et al, 1978], although ovalbumin does have a signal for secretion [Lingappa et al, 1979]. A long sequence of hydrophobic residues which span the bilayer could function as a stop transfer sequence such that the newly made protein remains associated with the membrane having its COOH-terminal end exposed to the cytoplasm and its NH_2-terminal on the opposite membrane face. This model is supported by many studies, including studies of the biogenesis of the histocompatibility antigens [Henning et al, 1976]; immunoglobulin μ-heavy chain [Singer et al, 1980; Alt et al, 1981; Rogers et al, 1980], glycophorin [Tomita et al, 1978; Tomita and Marchesi, 1975], the major coat protein of coliphage M13 [Wickner, 1975, 1976; Webster and Cashman, 1978], and vesicular stomatitis virus (VSV) G protein [Katz et al, 1977; Rothman and Lodish, 1977; Rothman et al, 1978; Toneguzzo and Ghosh, 1978]. However, other membrane proteins are more complex in their association with the lipid bilayer. For example, bacteriorhodopsin, a 27,000-dalton polypeptide, crosses the membrane several times as a folded α-helical rod [Henderson, 1975; Henderson and Unwin, 1975]. Similarly, protein I of the *Escherichia coli* outer membrane [Schindler and Rosenbusch, 1978; Chen et al, 1979; Nakae et al, 1979] band III protein [Drickamer, 1977, 1978; Steck et al, 1978; Fukuda et al, 1978; Rao, 1979], and the anion transport channel of human erythrocyte also crosses the bilayer several times. Furthermore, band III protein isomaltase [Semenza, 1979] and aminopeptidase [Wickner, 1980] have their NH_2-terminal ends facing the cytoplasm side and their COOH-terminal ends exposed on the opposite side of the membrane. While many integral proteins are initially synthesized with a leader sequence, others are made without one (ie, cytochrome P450 [Hangen et al, 1977], cytochrome b5 [Okada et al, 1979], Sindbis virus protein PE_2 [Bonatti and Blobel, 1979], and lens membrane protein MP26 [Ramackers et al, 1980]). One possible explanation for these observations is that these proteins must assemble into the membrane posttranslationally. The leader peptide allows the growing chain to fold in a manner compatible with the aqueous environment. Upon binding to the appropriate membrane, the protein interacts with lipid components to fold into a conformation that exposes hydrophobic residues to the bilayer fatty acyl chains. The NH_2-terminal end is then cleaved, rendering the process irreversible [Wickner, 1979]. This model could help explain how proteins are capable of crossing one membrane and specifically assembling into a second membrane (ie, the mitochondrial proteins cytochrome b5, NADH cytochrome b5, cytochrome c1 [Ross and Schatz, 1976], cytochrome c oxidase [Poyton and McKemmie, 1979a,b], and several subunits of the F_1 ATPase [Maccecchini et al, 1979]). The question arises whether proteins enter the membrane during their synthesis (ie, cotranslational assembly) or afterward (ie, posttranslational assembly). It is clear that there is no single answer to the question of how proteins assemble into membranes. No single model can explain why (1) some proteins have their COOH-terminal in the cytoplasm and their NH_2-terminal on the other side, while others have the opposite orientation; (2) some proteins span the bilayer more than one time; (3) some are synthesized with a leader sequence, others without one. However, from all of these types of studies, a characteristic behavior shared by all integral membrane proteins emerges. Since not all integral proteins contain a long sequence of apolar amino acid residues, the ultimate orientation and conformation of these proteins in the membrane probably depends on the location of one or more apolar residues within the polypeptide, which, brought together by the folding pattern of the protein, forms an apolar surface that will interact with the bilayer fatty acyl chains, thereby anchoring the protein in the lipid bilayer. Furthermore, there is growing evidence that proteins which transverse the membrane and are involved in one type of transport function or another exist in an oligomeric form with an axial transmembrane arrangement. The benefit these molecules de-

rive from their oligomeric structure is that they can (1) form a relatively rigid channel extending through the membrane to allow for nonspecific passage of solutes and/or (2) form a gated channel such that no instance do they freely extend across the whole membrane. Cross-linking experiments have shown that $(Na^+ + K^+)$ ATPase exists in the membrane as a dimer of the large α and β subunits, $(\alpha\beta)_2$. ADP/ATP carrier also exists as a dimer, α_2; and Ca^{2+} ATPase, as a tetramer $(\alpha)_4$. The acetylcholine receptor from electric organs of *Torpedo californica* (Torpedo) or *Electrophorus electrica* (eel) is composed of four glycopeptide subunits of Mr 40,000, 50,000, 60,000, and 65,000 [Conti-Troconi and Raftery, 1982]. These complex macromolecules contain two functionally distinct components, an acetylcholine-binding site and the Na^+-specific channel it regulates.

Other mechanisms exist for the transport of exogenous macromolecules from the cell environment at rates often many thousandfold above the rate of uptake by normal fluid-phase pinocytosis. These macromolecules bind specifically to the membrane at the cell surface and are interiorized by receptor-mediated endocytosis [Goldstein et al, 1979]. Cell membrane receptors have been identified, for example, for low-density lipoprotein (LDL) in human fibroblasts [Brown et al, 1981, Brown and Goldstein, 1979, Basu et al, 1978], for galactose-terminated glycoproteins on liver cells [Ashwell and Hartford, 1982], and for insulin on liver cells and fibroblasts [Schlessinger et al, 1978, Carpentier et al, 1979]. It is not yet clear with respect to most of these receptors whether they have oligomeric structure.

Glycosylation of Membrane Proteins

Another characteristic feature of membrane proteins is that many of them are glycosylated. Two classes of linkages between the oligosaccharide chains and the polypeptides backbone of the protein have been identified. They are N- and O-glycosidic linkages (Fig. 1).

In the case of N-glycosidic linkage, there can be polymannose chains such as those found on ovalbumin and the complex chains found in molecules such as IgM. This linkage is characterized by an enzymatic en block transfer of a complete oligosaccharide chain to an asparagine [ASn] residue that is part of the sequence Asn-X-Ser/Thr. However, not all Asn in this sequence are necessarily involved in glycosidic linkage. In contrast, O-glycosidically linked chains are characterized by a linkage between N-acetylglucosamine and a serine or threonine residue in the polypeptide. The molecular mechanism underlying this process is quite complex. In the case of N-glycosidic linkage, glycosylation of the protein occurs while the growing polypeptide chain is still nascent on the membrane-bound polysomes. The different steps involved are summarized below:

1) The initial stage of assembly involves the synthesis of a dolichol pyrophosphate oligosaccharide, probably in the membrane of the endoplasmic reticulum (Fig. 2).

2) Consistent with the assembly of the oligosaccharide-lipid, the protein destined to be glycosylated is inserted through the rough endoplasmic reticulum. Once the Asn residues on the growing polypeptide chain are accessible to the luminal face of the rough endoplasmic reticulum, an en block transfer of the oligosaccharide occurs.

3) Following this transfer, there is a series of processing or trimming steps by enzymes localized in the microsomal fraction of the cell consisting of rough and smooth endoplasmic reticulum and/or Golgi membranes [Hanover and Lennarz, 1981]. The final modification is believed to occur in the Golgi in which a high mannose-type structure has been transformed into one containing N-acetylglucosamine, galactose, sialic acid, fucose, and possibly others sugars (Fig. 3).

Glycosyl transferase involved in the attachment of the sugar moieties in the O-linked oligosaccharides are found in the Golgi apparatus. Study of the synthesis of glycophorin

[Jokinen et al, 1979], a membrane protein containing one N- and 15 O-linked chains, or chorionic gonadotropin (HCG) [Ruddon et al, 1980], a glycoprotein hormone that contains four O- and five N-linked oligosaccharides chains, shows that the events of O-glycosylation occur late in the assembly process, only after addition of the N-linked chains, presumably in the Golgi. Unlike N-glycosylation, O-glycosylation does not utilize the dolichol phosphate intermediate but follows the more classical glycosyl transfer reactions such as in the stepwise addition of galactose, N-acetylglucosamine, sialic acid, or fucose to glycoproteins. Furthermore, the amino acid residues involved in O-glygosyl linkage are often clustered and in proline-rich regions. No general "signal sequence" comparable to that found for N-glycosylation has been identified for O-glycosylation.

Subsequent steps following glycosylation involve packaging of the protein in secretory vesicles, in the case of secretory proteins, or in the membrane of presumptive vesicles and the fusion of these vesicles with the existing membrane, leading to deposition of the glycoprotein outside of the cell surface.

The mechanisms of migration of the newly synthesized proteins from the endoplasmic reticulum to the Golgi is not well understood. There is evidence suggesting that coated vesicles may be involved in the transport of the fully glycosylated VSV G protein to the plasma membrane [Rothman and Fine, 1980], but this evidence is indirect and not compelling.

A general pattern concerning the organization of protein in the lipid bilayer can be formulated. Proteins that are partially imbedded in the bilayer from the cytoplasmic or from

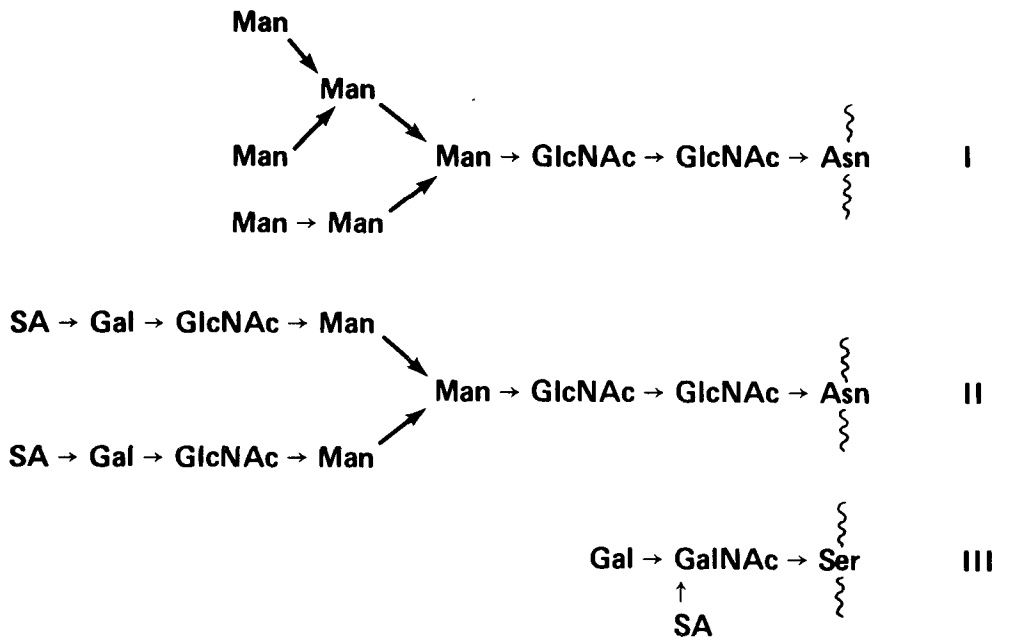

Fig. 1. *Typical structure of polymannose (I) and complex type (II) oligosaccharide chains N-glycosidically linked to an asparagine residue, as well as a typical oligosaccharide O-glycosidically linked to a serine residue (III) are shown. The position and anomeric configurations of the linkages are omitted for simplicity . (from Hanover and Lennarz [1981]).*